Case Report Rapport de cas

B-cell lymphoma in a dog with ehrlichiosis *(Ehrlichia canis)* and systemic histoplasmosis *(Histoplasma capsulatum)*

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Abstract — A mixed breed dog treated for ehrlichiosis and systemic histoplasmosis developed a refractory thrombocytopenia. When an abdominal mass was detected, exploratory laparotomy and biopsies confirmed lymphoma, which on immunohistochemical stains was determined to be of B-cell origin. Conceivably, the B-cell lymphoma in this dog was associated with chronic inflammation from ehrlichiosis, histoplasmosis, or both.

Résumé — Lymphome malin à cellules B chez un chien avec ehrlichiose *(Ehrlichia canis)* et histoplasmose systémique *(Histoplasma capsulatum)*. Un chien de race croisée traité pour ehrlichiose et histoplasmose systémique a développé une thrombocytopénie résistante. Lorsqu'une masse abdominale a été détectée, la laparotomie exploratrice et les biopsies ont confirmé la présence d'un lymphome. L'immunohistochimie a par la suite révélé qu'il provenait des cellules B.

Il est concevable que le lymphome malin à cellules B de ce chien soit associé à l'inflammation chronique causée par l'ehrlichiose, l'histoplasmose ou les deux.

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A 5-year-old, spayed female, mixed breed dog with a 1-month history of lethargy and weakness was referred to the Oklahoma State University Veterinary Teaching Hospital (VTH). A presumptive diagnosis of a rickettsial infection had been made by the referring veterinarian, based on thrombocytopenia on the hemogram, the prevalence of rickettsial diseases in Oklahoma, and the known tick vector exposure for this dog. The referring veterinarian had treated the dog with doxycycline (5 mg/kg body weight (BW), PO, q12h) and prednisone (1 mg/kg BW/d, PO) for 4 wk with only minimal improvement.

Case description

When referred for a 2nd opinion, the only abnormal finding on physical examination was splenomegaly. A persistent marked thrombocytopenia (35×10^9/L manual count; reference range, 200 to 500 $\times$ 10^9/L) was revealed on the hemogram (Abbott Cell-Dyne model 3500; Abbott Park, Illinois, USA) and a hyperglobulinemia (69 g/L, reference range, 25 to 39 g/L) was the only abnormality on the serum chemical profile (Vitros 250; Ortho-Clinical Diagnostics, Johnson and Johnson Company, Raritan, New Jersey, USA). Thoracic radiographs were normal; abdominal radiographs confirmed moderate splenomegaly and revealed concurrent mild hepatomegaly. Abdominal ultrasonography revealed no additional abnormal findings. Cytologic examination of bone marrow aspirates established the diagnosis of systemic *Histoplasmosis capsulatum* infection and revealed granulomatous inflammation, mild myeloid hyperplasia, moderate megakaryocytic hyperplasia, and moderate immune stimulation reflected by increased plasma cells. Itraconazole (Sporanox; Ortho Biotech Products, LP, Raritan, New Jersey, USA) at 5 mg/kg BW, PO, q12h, was initiated for a minimum of 60 d. An antibody titer for *Ehrlichia canis* by immunofluorescence antibody (IFA) was positive ($>$ 1:40); while antibody titers for *Rickettsia rickettsii* and *Borrelia burgedorferi* were negative. Positive titers for *E. canis* reflect prior exposure and titers can remain positive for many months after treatment with doxycycline (1–5); therefore, treatment with doxycycline was not reinstituted, because an appropriate course of therapy had previously been administered.

The dog returned to the VTH in 2 mo for a reevaluation that included a repeat bone marrow aspirate to evaluate the effectiveness of the antifungal treatment. The dog had improved clinically with itraconazole treatment. Hemograms repeated by the referring veterinarian had revealed improvement in the dog's thrombocytopenia ($\geq 185 \times 10^9$/L), but her automated platelet counts never increased to within the reference range. A manual count at the VTH revealed decreased platelets at 82×10^9/L and the serum globulins remained increased at 46 g/L. Cytologic examination of smears of a bone marrow aspirate now revealed mild erythroid and megakaryocytic hyperplasia; increased reactive plasma cells, consistent with immune stimulation; but no *Histoplasma* organisms or intracellular *Ehrlichia* morulae. Platelet surface-associated immunoglobulin

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(Ig) G by IFA (KSU Immunology Laboratory, Manhattan, Kansas, USA) in peripheral blood was negative, which did not support immune-mediated peripheral platelet loss. While no *Histoplasma* organisms were seen on cytologic examination of the bone marrow aspirate, oral itraconazole treatment was continued for an additional month.

The dog was reevaluated 1 mo after discontinuing oral itraconazole. The dog was normal on examination and without clinical signs, but a manual platelet count remained decreased at 129×10^9/L. Cytologic examination of bone marrow aspirates revealed normal cellularity and maturation of the megakaryocyte, erythroid, and myeloid cell lines, but no intracellular *Histoplasma* organisms or *Ehrlichia* morulae. No additional treatment was instituted and the owners were instructed that the dog was to be rechecked if any clinical signs returned. The dog was represented to the VTH 2 mo later with a distended abdomen and generalized mild peripheral lymphadenopathy. The dog was reported to have been mildly depressed and lethargic for approximately 2 wk, with soft stools and occasional vomiting within the last 24 h. Abdominal ultrasonography revealed moderate hepatosplenomegaly and mesenteric lymphadenopathy. A serum biochemical profile revealed hypoalbuminemia (18 g/L; reference range normal, 23 to 39 g/L) and hyperglobulinemia 44 g/L. A hemogram now revealed severe thrombocytopenia (16.3×10^9/L, by manual count) with marked leukopenia (2900×10^6/L; reference range 5000 to 17 000 $\times 10^6$/L) and decreased segmented neutrophils (2030×10^6/L; reference range 3000 to 12 000 $\times 10^6$/L). The red blood cell count and hemoglobin were within the normal reference range. An IFA titer for a possible *Ehrlichia platys*-related thrombocytopenia coinfection was negative (5,6). Cytologic examination of additional bone marrow aspirates revealed a high percentage of blast cells (10% to 15%); however, there was also an increase in promyelocytes, indicating that the marrow was likely recovering from an overwhelming peripheral demand for inflammatory cells, with subsequent depletion of the storage pool, as was reflected in the hemogram. A 2nd population of blasts that was observed represented the monocytic series; there was also an increase in reactive plasma cells. The erythroid series was present, although in low numbers, and since no anemia was present, this reflected a relative erythroid hypoplasia. No evidence of maturational abnormalities was noted in any of the cell lines, and no intracellular *Histoplasma* organisms or *Ehrlichia* morulae were seen. Cells in fine needle aspirate smears from prescapular lymph nodes were characterized as reactive, suggesting immune stimulation. Although antigen titers by an enzyme immunoassay (MiraVista Diagnostics, Indianapolis, Indiana, USA) and cytologic examination of rectal scrapes for *H. capsulatum* proved negative, oral itraconazole was reinstituted at the same dose and frequency, because *H. capsulatum* can establish a dormant phase when not totally eliminated from the mononuclear phagocyte system (7). When rechecked by the referring veterinarian, the automated platelet count increased, but never to within the reference range.

The dog returned to the VTH approximately 6 wk later for follow-up evaluation. The owner reported that the dog had improved slightly, with more energy, normal stools, and no vomiting. However, on physical examination, the left prescapular lymph node was now estimated to be twice the normal size. Abdominal palpation revealed hepatosplenomegaly and ascites, as well as a 10- to 12-cm, circular mass in the mid-abdomen. A hemogram confirmed a persistent thrombocytopenia 100×10^9/L by manual count with white blood cells 5000×10^9/L; 4200×10^9/L neutrophils, lymphopenia (600×10^9/L, reference range 1000 to 5000 $\times 10^9$/L), and monocytopenia (100×10^9/L, reference range 150 to 1300 $\times 10^9$/L). Abdominal ultrasonography confirmed the presence of a moderate amount of hypoechoic to anechoic peritoneal fluid and a large, echocomplex mass approximately 6.6×7.5 cm within the left mid-abdominal cavity, ventral and medial to the spleen. The grey-scale appearance and location of the mass appeared most consistent with that of an enlarged mesenteric lymph node. Cranial to this mass and adjacent to the aorta near the cranial mesenteric and celiac arteries, there were 2 additional well-defined, rounded, and hypoechoic masses, measuring approximately 3.4×3.9 cm, that also appeared to be large mesenteric lymph nodes. The splenic parenchyma contained multiple, small, round, increased echogenic lesions surrounded by hypoechoic rims (target lesions). Cytologic interpretation of fine needle aspirate smears from enlarged left prescapular and left submandibular lymph nodes now revealed that more than 90% of the cells were large basophilic lymphoblasts, suggestive of lymphoma.

An exploratory celiotomy was performed to investigate the possibility of resecting the large mass and obtaining biopsies from large mesenteric lymph nodes, spleen, and liver for histologic examination. The large mass was located at the root of the mesentery and was not associated with other organs. Because of its location, the mass was not resectable, but histologic examination of biopsies obtained from it and the enlarged mesenteric lymph nodes confirmed lymphoma. Immunohistochemical stains of sections of these lymph nodes revealed neoplastic cells that were strongly positive for CD79, a B-cell marker, supporting the diagnosis of a B-cell lymphoma (8). No evidence of lymphoma was seen on histologic examination of biopsies of the liver and spleen. No fungi were seen with periodic acid-Schiff staining; this was requested prior to instituting immunosuppressive chemotherapy for lymphoma. Repeat bone marrow aspirates also did not reveal any organisms or neoplastic cells, but there were increased numbers of megakaryocytes and granulocytes, primarily progranulocytes and myeloblasts, and scattered plasma cells on cytologic examination.

Chemotherapy was instituted 24 h postoperatively, according to the Madison-Wisconsin protocol (School of Veterinary Medicine, Madison, Wisconsin, USA), due to the dog's worsening clinical signs and the development of melena and dependent edema in all 4 limbs, the abdomen, and the thorax, as the edema was most likely associated with a lymphoma-induced vasculitis. A B-cell lymphoma-associated vasculitis or lymphatic obstruction was suspected (9). Over the next several days, the dog improved and was sent home to be treated with prednisone (Prednis-Tab, Vedco; Phoenix Pharmaceutical, St. Joseph, Missouri, USA), 1 mg/kg BW, PO, q12h, in accordance with the chemotherapy protocol. Enrofloxacin (Baytril; Bayer, Pittsburgh,

Pennsylvania, USA) 4.8 mg/kg PO, q12h, and famotidine (Pepcid AC; J and J Merck, Whitehouse Station, New Jersey, USA), 0.3 mg/kg BW, PO, q12h, which had been administered because of the melena, were also continued. The dog's platelet count at discharge had increased to 330×10^9/L.

One week later the dog received the 1st cyclophosphamide (Cytoxan; Bristol-Myers Squibb, New York, New York, USA) treatment (200 mg/mm^2, IV) for lymphoma, but she vomited and became extremely lethargic approximately 36 h after its administration. Unfortunately, the dog died at home a few hours later but was brought to the VTH for postmortem examination.

Gross postmortem examination revealed infarction of nearly the entire length of the small intestine, secondary to thrombosis in the cranial mesenteric artery just distal to its origin from the aorta. The thrombosis may have been secondary to stasis of blood flow in this region due to compression/distortion of the vessel by the adjacent lymphadenopathy and possibly a hypercoagulable state following immune-suppressive doses of corticosteroids. The large mass surrounding the cranial mesenteric artery appeared to be an extension of the regional lymphoma. In this dog, the definitive diagnosis of B-cell lymphoma was made, based on antemortem biopsies, and, at necropsy, there was no evidence of *Histoplasma* organisms in any of the tissues examined histologically.

Discussion

The etiology for the development of lymphoma in dogs is not well known. Possible environmental determinants include 2,4-dichlorophenoxyacetic acid (2,4-D), electromagnetic fields, and living in industrial areas (10). Genetic factors, such as mutations in the tumor suppressor gene p53, have also been reported to exist in dogs and may be involved in the known predisposition of some breeds to cancer (11). Also, various infectious agents have been associated with the development of malignancies in both humans and animals (8,12,13). Bacteria have been linked to development of cancer by 2 mechanisms: 1) induction of chronic inflammation, and 2) production of carcinogenic bacterial metabolites (14–17). An example is *Helicobacter pylori*, which has been associated with both adenocarcinoma in the pyloric part of the stomach and gastric B-cell lymphoma in humans (14–16,18–20) by inducing cell proliferation and the production of mutagenic free radicals and *N*-nitroso compounds, secondary to inflammation (14–17). Another bacterium-related lymphoma in humans is immunoproliferative small intestinal disease or Mediterranean lymphoma (19). When antibiotic treatment is instituted early, up to 40% will completely regress by an unknown mechanism (19).

Viral diseases have also been associated with malignancies (20). *Epstein-Barr virus* has been associated with Burkitt's lymphoma and other neoplasias in humans (12,18). *Herpes virus* induces Marek's lymphoma in chickens. *Maedi-visna virus* in goats, viruses causing malignant catarrhal fever in cattle, *bovine leukemia virus* in cattle, and retrovirus-induced oncornavirus cell membrane antigen lymphoma in cats are other examples of viral disease-associated malignancy in animals.

Immunosuppression resulting in decreased or impaired surveillance predisposes to the establishment and maintenance of malignancy (13). In immune suppressed humans, the increased prevalence of viral, bacterial, and protozoal infections seen with malignant B-cell lymphoma and, less commonly, T-cell lymphoma has been attributed to the constant antigenic stimulation and proliferation of mutated or transformed cells (13). Increased cell proliferation caused by any infectious agent may increase the risk of neoplastic transformation (14).

The dog of this report was *E. canis* antibody positive on referral and it is likely that the dog's infection may never have completely cleared with doxycycline treatment (1–4). Infrequently, a dog can maintain a persistent thrombocytopenia for years after antibiotic therapy (5). It is unclear whether this is due to a chronic *Ehrlichia* infection or infection with other pathogens, or if the persistent hematologic abnormalities are mediated through altered immunoregulation induced by the organism (5). Other possibilities for the persistent thrombocytopenia include immune-mediated destruction of platelets, splenic sequestration, and increased consumption. In this case, decreased bone marrow production, while possible, is less likely, as adequate to increased numbers of megakaryocytes were noted on multiple bone marrow aspirates. Interestingly, the only documented thrombocyte count within the normal reference range occurred after chemotherapy was instituted.

Chronic inflammation has been shown to induce plasma-cell proliferation (13) and development of neoplasia (3,14,15,17,18). Stimulation of a single clone of plasma cells also may result in a monoclonal gammopathy (3,21) with hyperglobulinemia. The altered immunity caused by *Ehrlichia* infections can result in dysregulation or disruption of the CD4:CD8 ratio by an as yet unknown mechanism (2). Unfortunately, electrophoresis was not done on sera saved from this dog, and flow cytometry to determine the CD4:CD8 ratio on fresh unclotted blood samples from this dog was not available at the time. Ehrlichiosis-related immunodysregulation (2) may have predisposed this dog to develop systemic histoplasmosis. Furthermore, the possibility of decreased B-cell surveillance and poorly regulated cell division could have permitted lymphoblast transformation and development of B-cell lymphoma. The authors propose that similar to the chronic inflammation associated with other infectious agents that have been linked to cancer formation (14–17,20), it is plausible that chronic inflammation related to either ehrlichiosis, systemic histoplasmosis, or both, may have been associated with subsequent development of B-cell lymphoma in the dog reported here. To the authors' knowledge, few papers exist reporting an association between rickettsial or fungal organisms and the development of neoplasia. Further research will be needed to elucidate the possible mechanisms of transformation to malignancy with these diseases. CVJ

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Book Review
Compte rendu de livre

Emerging Equine Science

Alliston J, Chadd S, Ede A, Hemmings A, Hylsop J, Longland A, Moreton H, Moore-Colyer M. Blackwell Publishing Professional, Ames, Iowa, USA, 2004. 254 pp. ISBN 1-8976-7647-6.

This book is a compilation of papers and posters given at a combined meeting of the British Society of Animal Science and the British Grasslands Society held at the Royal Agricultural College, Cirencester, UK in September 2003. The forward written by HRH the Princess Royal recognizes the importance of the Equine Industry in the UK and acknowledges the need for a better understanding of equine husbandry and health management. The book includes 2 parts. The first part is comprised of 16 chapters, essentially review papers. The second part includes 25 research posters.

Chapters 1–3 are devoted to muscle physiology and locomotion and provide a concise, though somewhat superficial coverage, of muscle fiber types and response to exercise. Chapters 4, 7, and 14 are reviews of respiratory physiology and pathophysiology with specific reference to ventilation and inflammatory markers. Chapters 5 and 6 are concerned with stereotypic behavior. Chapters 8–10 provide brief coverage of modern breeding technologies, including a paper on the mapping of the gray gene in Thoroughbreds. With the exception of Chapter 16 which provides an excellent review of the challenges of attracting funding for equine events, the remaining chapters provide a review of gastrointestinal physiology and nutrition. Overall, these chapters are well written, well referenced and provide a good starting point for the novice.

The second part of the book is comprised of 25 research posters, most of which describe research in nutrition and behavior. Behavior posters emphasize stereotypy. One notable poster details the use of in-stall mirrors as in aid in the management of stereotypic behaviors.

This is more of a "proceedings" than a book. Animal science students and veterinary students interested in either equine nutrition or stereotypic behavior will find it a useful introduction. Experienced nutritionists and behaviorists would appreciate the poster section.

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